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Assessment of Drugs of Abuse in a Wastewater Treatment Plant in New Zealand with Parallel Secondary Wastewater Treatment Train

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Abstract

In this study, 24-hour composite wastewater samples were collected from a wastewater treatment plant of New Zealand with parallel secondary treatment units. The aim was to investigate occurrence, removal, and consumption of 13 drugs of abuse (DOAs) including illicit drugs, alcohol, nicotine, and their metabolites. The filtered samples were analyzed through direct injection on LC-MS/MS. Ethyl sulfate, one of the major metabolites of alcohol, was detected at highest concentration (mean = 8,300 ng/L) in wastewater influent. The mean concentrations of methamphetamine and hydroxycotinine in the influent were found to be 935 ng/L and 5,000 ng/L, respectively. Amphetamine (383 ng/L) and cocaine (286 ng/L) were detected at highest concentrations in the effluent. The removal efficiency of the treatment plant varied for DOAs: >99% for morphine, ethyl sulfate, and hydroxycotinine and <50% for methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). Primary treatment did not show any significant removal of DOAs while the removal efficiencies of total monitored DOAs by Membrane Bioreactor (MBR) and Bardenpho processes were found to be similar (~95% removal). The population was estimated using hydrochemical parameters and human urine biomarkers and showed good agreement with wastewater treatment plant's estimates. Weekday-weekend variation in the consumption of alcohol and methamphetamine was found to be significant, with a higher estimated consumption during the weekends. Monitored DOAs in influent were present at highest concentrations during summer (23 µg/L), at low concentrations during winter (17 µg/L), and at lowest concentrations during heavy rainfall event (11 µg/L), possibly due to dilution. The population normalised mass loads of DOAs were found to correlate with their metabolites, and morphine was found to correlate with nicotine metabolites.

Keywords: illicit drugs; wastewater; occurrence and fate; alcohol; nicotine; parallel secondary treatment

1. Introduction

The United Nations Office on Drugs and Crime (UNODC) has reported that an estimated 5.2% of the world's population aged 15-64 used illicit drugs in 2014 (UNODC 2016). The traditional self-reported survey methods do not always give an accurate estimate of illicit drug consumption in a community (Metcalfe et al. 2010). Wastewater based epidemiology (WBE) is increasingly used as a method to estimate the quantity of drug consumption in communities and can complement to the existing epidemiological surveys and illicit drugs seizure data (McCall et al. 2016, Yadav et al. 2017, Yargeau et al. 2014, Zuccato et al. 2008). Illicit drugs and their metabolites in wastewater influent have been reported to be in the range of <1 ng/L up to 10 µg/L (Yadav et al. 2017). These DOAs and their metabolites have also been detected in surface water ranging from sub-ng level to >100 ng/L, due to their inefficient removal during wastewater treatment (Yadav et al. 2017). The occurrence of these drugs, even at sub-µg/L concentrations in the aquatic ecosystem, may produce ecotoxicological effects (Pal et al. 2013).

The removal efficiencies of DOAs in a wastewater treatment plant (WWTP) are still not well known, especially with advanced treatment processes such as membrane bioreactors (MBR) (Evgenidou et al. 2015, Kim et al. 2014). Although few studies have assessed removal efficiencies of DOAs by WWTPs (Andrés-Costa et al. 2014, Kasprzyk-Hordern et al. 2009, Postigo et al. 2010, Terzic et al. 2010), more research is needed to assess and compare their removal through conventional and advanced treatments. Furthermore, in New Zealand, the previous studies have mainly focussed on DOAs' consumption, based on catchment population

data given by WWTP operators and wastewater influent concentrations only (Lai et al. 2017). There is a lack of information about occurrences of DOAs in wastewater effluents; such information is particularly relevant as practices of effluent application to land or its discharge to surface water bodies are not uncommon.

Community drug use can be estimated by measuring the concentration of drug target residues (DTR) in the wastewater influent (Baker et al. 2014, Chen et al. 2014, Gatidou et al. 2016, Jones et al. 2014, Mastroianni et al. 2016, van Nuijs et al. 2011). Numerous WBE studies have been conducted across the globe, including in European Union, U.S., Australia, and Asia, as reported by Yadav et al. (2017) and in New Zealand (Lai et al. 2017). The Sewage Analysis Core Group Europe (SCORE) also performs interlaboratory sewage analysis across Europe, Australia, and North America to estimate the selected drug residues in wastewater influent (van Nuijs et al. 2018).

Apart from the illicit drugs, the WBE method can also estimate alcohol consumption of the population, served by a specific sewerage system, by quantification of the mass load of a stable and specific biomarker of its oxidative metabolism (Andrés-Costa et al. 2016, Boogaerts et al. 2016, Ryu et al. 2016). Similarly, nicotine consumption in the community can also be monitored by near real-time sewage analysis (Castiglioni et al. 2015). WBE study not only reveals the consumption pattern of drugs but can also be used to estimate the *de facto* population of the catchment area served by a wastewater treatment plant by measuring the mass load of relatively stable biomarkers, taking into account average human excretion rate (Daughton 2012, O'Brien et al. 2014, Senta et al. 2015).

Consumption of some of the drugs like methamphetamine, cocaine, and MDMA has been estimated using WBE in New Zealand (Lai et al. 2017); however, the consumption patterns of

most of the other DOAs, including alcohol and nicotine, has not been reported and hence, this study will also contribute to the existing community drug consumption pattern derived from epidemiological surveys. The social cost (includes cost of personal and community harm and intervention costs) associated with consumption of illicit drugs in New Zealand is estimated to be 1.8 billion per year(McFaddenConsultancy 2016). The estimated social cost associated with consumption of alcohol and nicotine is higher than illicit drugs in both Australia and New Zealand. Hence, it will be relevant to estimate consumption patterns of DOAs in this region using WBE approach.

The principal aim of this study was to investigate the occurrence and removal of DOAs in an urban wastewater treatment plant in New Zealand by analysing DOAs at different stages of wastewater treatment in four seasons. The uniqueness of the study lies with the seasonal sampling at different stages of the WWTP, which had a parallel secondary treatment train, with conventional and advanced treatment components. Hence, the study yields comprehensive data on the occurrence and removal of DOAs in the studied WWTP. Consequently, the influent and effluent mass loads of DOAs were used to estimate the population consumption of DOAs and their environmental discharges, respectively.

2. Material and Methods

2.1. Chemicals and consumables

Chemicals, including isotope labelled standards, used in this study were of analytical grade and purchased from Cerilliant (USA). Methanol and acetonitrile (LC grade) were purchased from Merck (Germany). Formic acid was purchased from Sigma Aldrich (Australia). HPLC columns were purchased from Phenomenex (USA). The DOAs selected for the study (**Table S1**) were methamphetamine, amphetamine, cocaine, nicotine, codeine, 3,4-methylenedioxy

methamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-n-ethylamphetamine (MDEA), morphine, methadone, ketamine, methylone, oxycodone, mephedrone, and buprenorphine, whereas metabolites included benzoylecgonine, cotinine, hydroxycotinine, ethyl sulfate, norketamine, and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). Native and labelled analytical standards of these DOAs and their metabolites were obtained from various suppliers.

Table S1 lists the 21 DOAs and DTRs, along with their physiochemical properties, chosen for the study. DOAs were selected based on their global occurrence in wastewater (Pal et al. 2013) and compatibility with existing analytical methods.

2.2. Sampling

Wastewater samples were collected from an urban WWTP in New Zealand, serving a catchment area population of less than 100,000. This catchment area was selected for the study because it has been a major tourist destination in New Zealand for more than 100 years and was not part of the previous studies. The selected WWTP received mostly domestic wastewater with a minor contribution coming from industries. The main sources of trade waste were waste management, paints industry, dry cleaners, and meat processing plant. The sources of wastewater are included in **Table S2**.

The process scheme **(Figure 1)** included preliminary treatment (screens and grit removal), primary treatment (sedimentation), and secondary treatment. A secondary treatment process consisted of two parallel units: Bardenpho (conventional activated sludge coupled with nutrient removal) and Membrane Bioreactor (MBR), operating simultaneously and discharging their effluent to the forest for irrigation purpose. The Bardenpho system is a 5-stage biological treatment unit consisting of anaerobic/ anoxic/ aerobic/ anoxic/ aerobic zones, with target

MLSS of 4,000 mg/L, HRT of 13.5 hours, and sludge age of 12 days; whereas MBR is the integration of membrane filtration with biological secondary treatment, with target MLSS of 5,000 mg/L, HRT of 19.6 hours, and sludge age of 20 days. Based on the average flow data supplied by the WWTP, approximately 75% of primary effluent was treated by the Bardenpho process and remaining by the MBR. Secondary effluents from both treatment units combine to yield final effluent.

The raw and treated 24-hour composite wastewater samples of 5 litres each were collected daily in time proportional sampling mode, every 15 minutes over 24 hours, during a selected week in spring (October, 2016), summer (January, 2017), autumn (May, 2017), and winter (July, 2017) using ISCO autosampler (Teledyne, U.S.). Additional composite wastewater samples were also collected during a heavy rainfall period of consecutive four days in April 2017 to evaluate the effects of heavy rainfall on concentrations and fate of DOAs. The sampling details are provided in **Table S3**.

2.3 Wastewater quality characterisation

Wastewater samples were analysed by the WWTP within 24 hours according to the Standard Methods (Rice et al. 2017). WWTP analysed parameters included pH, alkalinity, carbonaceous biochemical oxygen demand (cBOD), chemical oxygen demand (COD), dissolved reactive phosphorus (DRP), nitrogen (includes ammonia, nitrite, total Kjeldahl nitrogen, total oxidised nitrogen), total phosphorus, turbidity, and suspended solids.

2.4 Sample processing

All samples were acidified to pH 2 at the WWTP immediately after sampling. The samples were then put on ice and transferred overnight to the Environmental Engineering laboratory of the University of Auckland. 20 mL of collected samples were filtered with 0.2 µm PTFE

syringe filter to eliminate suspended particles. pH of all the samples was verified with Thermo Scientific Orion 3-Star pH meter. Acidified and filtered wastewater samples were then sent to the University of Queensland (Australia) for analysis of the DOAs using high-performance liquid chromatography (Shimadzu Nexera UHPLC system, Kyoto, Japan) coupled to a triple quadrupole tandem mass spectrometer (AB SCIEX QTRAP®5500, Ontario, Canada).

2.5 LC-MS/MS analysis

Analysis was carried out using direct injection of samples on above-mentioned liquid chromatography tandem mass spectrometer (LC-MS/MS), thus avoiding any additional sample preparation and extraction steps. Samples were spiked with deuterated standards before analysing on LC-MS/MS for quality assurance and quality control (QA/QC) check. Phenomenex Biphenyl (50x2 mm, 2.6 micron) and Phenomenex Kinetek EVO C18 (50x2 mm, 1.7 micron) LC columns were used for the separation of DOAs and ethanol metabolites, respectively. LC-MS/MS parameters are shown in **Table S4**. MS acquisition parameters for each DOA are detailed in **Table S5**. Other QA/QC measures were implemented in the similar way as mentioned by Lai et al. (2013b) to ensure accurate quantification of selected DOAs.

The samples and MilliQ water laboratory blanks acidified to pH 2 (n = 3) were spiked with mass labelled internal standards to account for matrix effects. A six-point calibration standard curve was prepared in MilliQ water acidified to pH 2 and ranged in concentrations from 0.1 to 40 μg/L. The calibration curve (n = 6) and wastewater samples spiked with native standards (5 μg/L; n= 2) were analysed in duplicate and the relative differences were minimal. The limit of detections (LOD) for chemicals ranged from 0.003–0.2 μg/L (Table S6). No analytes were detected in blank samples. The analytical method is validated yearly through an inter-

laboratory comparison (SCORE 2015). More details of the analytical method are described in Text S1 and previous studies (O'Brien et al. 2014, Banks et al. 2018, Lai et al. 2017).

2.6. Estimation of removal efficiency

The removal efficiency was calculated based on following equation:

Removal efficiency of treatment unit (%) = [(Average seven days influent concentration to WWTP - Effluent concentration for Treatment Unit)/ Average seven days influent concentration to WWTP *100] **Equation (1)**

2.7 Estimation of mass loads and consumption of DOAs

The influent mass loads for DOAs were calculated by multiplying the concentration of DOAs with the daily inflow to WWTP. The population normalised mass load was calculated by dividing the daily mass load of each DOA with daily population estimate. The consumption of selected pharmaceuticals (mg/d/1000 person) was estimated by multiplying the population normalised mass load of DOAs with respective correction factors, which take into account the excretion factor and the ratio of molecular weight of parent drug and DTR (**Equation 2)** (Boogaerts et al. 2016, Castiglioni et al. 2015, Lai et al. 2013a, Mastroianni et al. 2017). For methamphetamine, cocaine, nicotine, alcohol, and MDMA, DTRs used were methamphetamine, benzoylecgonine, cotinine+hydroxycotinine, ethyl sulfate, and MDMA, respectively. The corresponding correction factors were 2.3 (van Nuijs et al. 2011), 2.33 (Castiglioni et al. 2015), 1.35 (Nefau et al. 2013), 3,047 (Boogaerts et al. 2016), and 1.5 (Castiglioni et al. 2015), respectively. The correction factor of 3,047 and alcohol density of 789 kg/m^3 were used to estimate the daily consumption of pure alcohol on a volume basis (Rodríguez-Álvarez et al. 2015, Yin et al. 2018).

Estimated drug consumption = (Concentration of DOAs (mg/L)* Flow (L/d)* Correction factor)/Population of catchment area derived from WBE*1000 **Equation (2)**

The amount of nicotine absorbed (g/day) by human body during smoking was estimated through the equation used by Castiglioni et al. (2015). The total nicotine absorbed was based on the sum of the mass loads of cotinine and hydroxycotinine, obtained by back calculation of their concentrations in ng/L, multiplied by correction factor of 1.35, as shown below in Equation 2:

Nicotine (absorbed) = [(Concentration$_{cotinine}$*F) + (Concentration$_{hydroxycotinine}$*F)]*Correction factor **Equation (3)**

2.8. Estimation of the population served by the WWTP

Population size was estimated using five hydrochemical parameters of wastewater: BOD, COD, TKN, TP, and ammonium (Chen et al. 2014, Rico et al. 2017, Senta et al. 2015). The population served by the WWTP was estimated by dividing the mass load of these parameters with their literature-reported wastewater per capita load. Mass load of BOD was divided by 60, COD by 128, Ammonia by 8.1, TKN by 10, and TP by 1.7 (Been et al. 2014, Rico et al. 2017).

Population size was also estimated using human urine indicators like methadone and codeine as follows:

Estimated population = (Daily mass load * Excretion factor)/Daily defined dose (DDD) per thousand **Equation (4)**

DDD per thousand of methadone and codeine were 1.91 mg and 64 mg, respectively (Ministry of Health 2018). The excretion factors used for codeine and methadone were 30% and 27.5% (Thai et al. 2016).

Population estimate by nicotine metabolites was calculated similarly. Nicotine is metabolised to cotinine, which is hydroxylated to hydroxycotinine along with other metabolites through enzymatic transformation (Buerge et al. 2008, Rodríguez-Álvarez et al. 2014). These biomarkers are excreted along with the fraction of unmetabolized nicotine in urine, after tobacco consumption. The number of cigarettes smoked in a day can be calculated based on the assumption that 1.25 mg of nicotine is absorbed while smoking one cigarette (Castiglioni et al. 2015). The average number of current smokers aged 15 years and more in the study area was reported to be 20.2% by the local district health board. Population was estimated considering that 76.8% of population were adults (age >15 years) in the study area, based on the national census of 2013. Similar approach of population estimates using nicotine was also done by Senta et al. (2015).

2.9 Statistical Analysis

The statistical analysis was carried out using IBM SPSS™ statistics 24. The Shapiro-Wilk test was performed to check if the sample has normal distribution of data. Parametric one way ANOVA followed by Tukey HSD and non-parametric Kruskal-Wallis test was performed to evaluate the statistical differences between different population estimates and their seasonal and weekday-weekend variation. Seasonal and weekday-weekend variations of DOAs consumption were also evaluated similarly. Non-parametric Spearman's rank correlation analysis was conducted in order to correlate population normalised mass load of DOAs.

3. Results and Discussion

3.1. Occurrence and Removal of DOAs in the WWTP

3.1.1. Concentration of DOAs in wastewater influent

Influent and effluent concentrations of DOAs and their comparison with international studies are summarised in **Table 1.** Out of 21, only 13 monitored DOAs were detected in the influent with the concentrations ranging from sub-ng/L to 15 µg/L. For calculating mean, median, and SD, <LOD values were excluded; however, mean and median were not calculated for compounds detected in only one season in the effluent. Overall, ethyl sulfate was present at highest concentration (mean = 8,300 ng/L), whereas MDMA (mean = 24 ng/L) was detected at lowest concentration among all detected DOAs. Ethyl sulfate and ethyl glucuronide are the two major metabolites of alcohol metabolism. Ethyl sulfate is a relatively stable and is a specific biomarker for alcohol consumption (Ryu et al. 2016, Wurst et al. 2006) while ethyl glucuronide is not stable. MDEA (<10 ng/L), ketamine (<10 ng/L), norketamine (<10 ng/L), methylone (<10 ng/L), mephedrone (< 30 ng/L), oxycodone (<20 ng/L), and buprenorphine (<50 ng/L) were not detected in any samples using the direct injection analytical protocol followed in this study. It was found that detection frequency was more than 90% for the majority of the detected DOAs, including DTRs. However, cocaine, benzoylecgonine, and MDMA were detected with frequency less than 15%. The mean concentrations of cotinine and hydroxycotinine were found to be 1,800 ng/L and 5,000 ng/L, respectively, while the mean concentration of nicotine was 3,000 ng/L. As can be seen from **Table 1**, most of the DOAs' concentrations were in a similar range when compared to studies conducted elsewhere.

The concentrations of methamphetamine, amphetamine, benzoylecgonine, ethyl sulphate, and MDMA were also compared with their respective concentrations in Australian wastewater influent. The median concentration of amphetamine and methamphetamine in this study was found to be significantly less than those reported by Gao et al. (2018b) in urban wastewater treatment plant of Australia. The WBE studies and seizure data have demonstrated that amphetamine consumption is prominent in Western Europe, whereas Northern Europe,

Slovakia, Czech Republic, and Oceania regions are dominated by methamphetamine consumption (Irvine et al. 2011, Ort et al. 2014). Amphetamine concentrations observed in this study might be due to methamphetamine metabolism in human body, causing it to appear in urine. However, amphetamine is also prescribed in NZ for therapeutic use. Amphetamines pills are sold as diet pills under the name of Duromine® and also as dexamphetamine (Dexedrine®, Dextrostat®) (MinistryofHealth 2010). The mean amphetamine/methamphetamine ratio was found to be 0.143 indicating methamphetamine consumption as a major source of amphetamine occurrence in these samples (Gao et al. 2018b). The mean concentration of ethyl sulphate in this study was found to be half of the concentration reported by Nguyen et al. (2018) in Australia. The mean concentration of MDMA and benzoylecgonine in this study were also found to be less than reported by Irvine et al. (2011) in Australian wastewater treatment plant.

The seasonal variation in mean concentration of DOAs in influent is shown in **Table 2**. Most of the drugs did not show seasonal variations. Cocaine was detected in only summer and winter seasons whereas MDMA was also present in only spring and summer seasons above LOD. Overall, for monitored DOAs, total concentrations were found to be 30-40% higher during summer than winter, possibly indicating the influence of tourists and increased recreational activities during summer season. Temperature variations were not expected to affect the stability of most of the DOAs (Senta et al. 2014), and hence, those were not expected to contribute to seasonal variations. However, recent study (Ramin et al. 2018) indicates that DOAs demonstrate increasing transformation rates with increasing temperatures of wastewater, and thus DOAs' influent mass loads reported in summer could be underestimated. Total concentrations for monitored DOAs were lowest, less than 50% of summer concentrations, during heavy rainfall event, which was expected due to significant dilution of

wastewater influent by runoff. **Table S7** in the supplementary information shows the seasonal variation of water quality parameters in influent.

3.1.2 Concentrations of DOAs in wastewater effluent

Codeine was present in wastewater effluent with mean concentrations of >100 ng/L. The mean concentrations of cotinine and methamphetamine were found to be in range of 50-100 ng/L while the mean concentrations of methadone and EDDP ranged between 0-50 ng/L in the effluent. Amphetamine (383 ng/L), cocaine (286 ng/L), benzoylecgonine (85 ng/L), and MDMA (20 ng/L) were detected in only one season in the effluent. Morphine, ethyl sulfate, hydroxycotinine, and nicotine were not detected in any seasons in the wastewater effluent. The standard deviations were significantly high in effluent samples for DOAs because of seasonal variation in their removal. Seasonal variations in DOAs' mean concentrations in secondary effluent and final effluent are shown in **Table S8** and **S9,** respectively. Mean removal efficiency of DOAs through parallel secondary treatment train of MBR and Bardenpho is shown in **Table S10**. Total population normalised effluent mass load of DOA was found to be 218 mg/d/1,000 population. The average effluent population normalised mass load (PNML) of DOAs is shown in **Table S11**. The seasonal variation in water quality parameters in final effluent is shown in **Table S12** while the seasonal variations in removal efficiency of water quality parameters is shown in **Table S13**.

3.1.3 Removal efficiency of WWTP

3.1.3.1. Primary treatment

The average removal efficiency of primary treatment in all four seasons was found to be insignificant (<1%) for majority of the drugs and their metabolites. Similar insignificant removal of DOAs at primary treatment was also reported by Subedi and Kannan (2014) at a

wastewater treatment plant in the U.S.A. The removal efficiency of primary treatment during a heavy rainfall period was found to be slightly better for most of the studied drugs and their metabolite (data not shown). One of the possible explanations is a higher amount of silt present during heavy rainfalls, which settles out in the primary treatment, removing with it the sediment-bound DOAs. Inefficiency of primary treatment indicates that sedimentation alone is not an efficient removal mechanism for most of the monitored DOAs.

3.1.3.2. Secondary treatment

MBR and Bardenpho were two parallel secondary treatment units of the studied WWTP. They operate simultaneously, and their effluent is mixed in an equalisation basin before discharge to the environment. The average removal efficiency of hydroxycotinine, nicotine, ethyl sulfate, and morphine was more than 99% for both MBR and Bardenpho. Negative removal efficiency was observed for amphetamine at Bardenpho during summer (**Tables S8**). Negative removal efficiency can be explained by the deconjugation of glucuronides metabolites in the secondary treatment process (Subedi and Kannan 2014). The higher effluent concentration of amphetamine could be because of transformation of methamphetamine to amphetamine during secondary treatment (Heuett et al. 2015).

The average methamphetamine removal was around 80% by MBR; however, complete removal was achieved by Bardenpho treatment. Average removal efficiencies of cotinine by MBR (95%) and Bardenpho (97%) were similar. Similarly, average removal efficiencies of codeine by MBR and Bardenpho were 83% and 87%, respectively. Average removal efficiency of methadone by MBR was found to be 53%, around four times higher than Bardenpho. Similarly, MBR was more efficient in removing EDDP, compared to Bardenpho. This may be

due to higher hydrophobicity of these compounds as MBR has greater bacterial floc density, which can assimilate hydrophobic compounds more efficiently.

Overall, secondary treatment was effective in removing 95% of the DOAs' mass load. Average removal efficiency was more than 99% for morphine, ethyl sulfate, and hydroxycotinine, irrespective of seasons. Average removal of methamphetamine, codeine, and cotinine was found to be >80%. Average removal of methadone and EDDP ranged between 20-30%. Average removal of methamphetamine, cotinine, and nicotine was of same order as obtained by Subedi and Kannan (2014) in the U.S. study. Negative removal of amphetamine has also been reported by Terzic et al. (2010). Cocaine, benzoylecgonine, and MDMA removal was higher than the reported removal efficiencies from several studies compiled in a review article by Yadav et al. (2017). Average removal efficiencies of secondary treatment unit, comprising of both MBR and Bardenpho, are shown in **Figure 2**.

The average removal efficiency of the WWTP was more than 90%, irrespective of seasons. This study also showed that there was insignificant difference (<5%) between removal efficiencies of MBR and Bardenpho for total concentration of monitored DOAs. The information was particularly relevant to the treatment plant as MBR installation was considered as an upgrade to remove trace level contaminants. Secondary treatment has been shown to be most effective for removal of DOAs in limited number of studies conducted on fate of illicit drugs in WWTP (Yadav et al. 2017). For example, differences observed in removal efficiencies of MDMA were solely attributed to differences in secondary treatment of studied WWTPs (Andrés-Costa et al. 2014). Although MBR is considered advanced wastewater treatment and is expected to perform better in terms of removal efficiencies for conventional parameters, activated sludge has also shown to be very efficient process for removal of DOAs (Yadav et al. 2017). Bardenpho, which is a modified form of activated sludge, has the same advantages

where contaminants can get volatilised due to aeration and settled out with waste activated sludge, in addition to being biodegraded. These are the possible reasons for a comparable performance of Bardenpho to that of MBR observed in this study.

There was insignificant variation (<5%) in Bardenpho removal of methamphetamine, morphine, ethyl sulfate, nicotine, cotinine, and hydroxycotinine in all seasons. Removal efficiency for amphetamine was found to be insignificant/negative during summer. Methadone removal was higher in summer and spring seasons, but substantially lower in autumn and winter seasons. Similarly, EDDP removal was higher in summer and autumn, compared to spring and winter season. Overall, summer samples did not show particularly high removal of total DOAs. This was contrary to most of the findings reported in the literature as the removal of organics during biological treatment is expected to be higher during warmer conditions. However, a rainfall event during our summer sampling week may have contributed to lowering of the temperature of wastewater and biological activity.

There is no information about MBR removal efficiency in spring season due to sample loss in transportation, so Bardenpho removal in spring was treated as the average DOA removal from secondary treatment in spring. Overall, there were no distinct variations (<5%) in removal of amphetamine, morphine, ethyl sulfate, nicotine, cotinine, cocaine, benzoylecgonine and hydroxy cotinine in three seasons at MBR. The removal efficiency of methadone, codeine, and methamphetamine was highest in autumn season (**Table S10).** The removal of EDDP was higher in winter and autumn compared to summer season. Lack of seasonal variations for removal of total DOAs mass load in MBR is consistent with the similar observation noted for Bardenpho.

3.2. Population estimates, Mass loads, and Consumption

3.2.1 Estimation of population size

Population size of the catchment was estimated using various hydrochemical parameters like COD, total phosphorus, cBOD, ammonium, and total nitrogen, and human urine indicators like methadone, codeine, cotinine, and hydroxycotinine. However, only nicotine marker was considered in this study for estimation of drug consumption.

3.2.1.1 Based on hydrochemical parameters

The mean population estimated by COD and total phosphorus were 67,845 and 62,014, respectively, which were in good agreement with population of 68,000 provided by wastewater treatment plant. The population was underestimated using cBOD (53,488) and overestimated using ammonium (96,325) and total nitrogen (107,381), and therefore, these parameters were not considered for estimating drugs' consumption. There was no significant difference found between weekday and weekend population ($p>0.05$). There was no significant seasonal difference of population estimated using mass load of COD ($p>0.05$) either; however, population estimation using total phosphorus showed significant seasonal variations **(Table S14)**, which could be attributed to phosphorus contribution through non-domestic sources of wastewater**.** Similarly, overestimation of population during heavy rainfall period by using COD is suspected because of organics washed in stormwater runoff. Hence, these hydrochemical parameters, although their estimates were in good agreement with WWTP estimate, were not used for estimation of consumption of DOAs in this study because of possible non-human sources of contribution to the wastewater (Senta et al. 2015).

3.2.1.2 Based on human urine indicators

Human urine biomarkers like cotinine, hydroxycotinine, methadone, and codeine were used to estimate the population per day. Average 70% of daily wastewater inflow (domestic inflow),

as communicated by WWTP operators (**Table S2)**, was used to calculate population estimate. The population estimated by nicotine metabolites and methadone had shown good agreement with each other ($p>0.05$) as well as with the population estimated by WWTP. However, only nicotine metabolites were used as human urine biomarker for estimation of population. Methadone and codeine were not considered because of an extreme outlier in the case of methadone **(Figure 3)** and possibility of illicit use of codeine causing underestimation of catchment area population. There was no significant difference found between weekday and weekend population estimates as well as between different seasons ($p>0.05$). Complete population estimates by hydrochemical parameters and human urine indicators are shown in Table **S14.**

3.2.2 Population normalised mass load and consumption

The average daily PNML of DOAs in the wastewater influent is shown in **Figure 4.** The seasonal variation in influent PNML of DOAs is shown in **Table S15.** The PNML of cocaine was found to be significantly lower than Europe wide study (75.89 mg/d/1000 people-821.7 mg/d/1000 people) of similar population range (ranging from >50,000 to <100,000) reported by EMCDDA (2017), except methamphetamine. The PNML of methamphetamine in this study was more than 20 times higher than Switzerland catchment with similar population range. However, the PNML of MDMA and amphetamine was found to be similar to PNMLs reported for catchments in France, and Switzerland and Belgium, respectively. The PNML of amphetamine was significantly lower than the PNML reported for a catchment in Iceland (169.9 mg/d/1000 person). The average number of cigarettes smoked was estimated as 10,150±2,194 per day based on the nicotine data (Castiglioni et al. 2015).

The average daily population normalised mass loads of ethyl sulfate was 1,883 mg/d/1000 population. Therefore, the average daily alcohol consumption was found to be 7.3 L/d/1000 person, which was significantly less than the mean alcohol consumption of 20.6 L/d/1000 population of 20 cities, including European, Australian and Canadian communities as studied by Ryu et al. (2016). The consumption of alcohol in this study was also found to be significantly less than national average of per capita alcohol consumption in New Zealand (~18 mL/person/day) (StatsNZ 2017), which could be because discrete areas may have very different consumption patterns, which require further investigation. A recent study by (Banks et al. 2018, Gao et al. 2018a) suggested that in sewer degradation of alcohol markers could be the reason for significant difference in per capita alcohol consumption estimated through WBE and epidemiological survey.

The average daily consumption for methamphetamine and codeine was 484±73 mg/d/1000 person and 241±26 mg/d/1000 person. The average daily consumption of methamphetamine, cocaine, MDMA, and alcohol was comparable to an Australian rural and urban community with a similar population (<150,000) (Lai et al. 2016, Yin et al. 2018). The average consumptions of DOAs estimated from this study were compared with wastewater influent studies done in New Zealand by Chappell et al. (2017) and Lai et al. (2017) (**Table 3)**. The methamphetamine and methadone consumption was found to be similar to Auckland; however, cocaine consumption was found to be highest compared to all the three cities. MDMA consumption was significantly lower than Auckland and Christchurch but higher than Whangarei consumption.

The consumption of methamphetamine exhibited seasonal variation (**Table S16**), with highest consumption in a week of the winter season ($p<0.05$). There could many reasons for spikes in methamphetamine use at a site including one-off entertainment events such as rock concerts,

sports events, and social gatherings (motorcycle club tours), but this was not explored in the current study. However, consumption of alcohol, codeine, and methadone did not show any seasonal pattern ($p>0.05$). The consumption of alcohol and methamphetamine exhibited weekday-weekend variation in spring and winter season (**Table S16**), with higher consumption on weekends ($p<0.05$). The cocaine and MDMA consumption did not show significant weekday-weekend variation because most of the values were close to LOD. The weekday-weekend consumption pattern was not observed for the remaining drugs in any season.

3.2.3 Limitations of the present study

The limitations of this study include unaccounted uncertainties due to sampling, in sewer stability of drug residues, and literature-reported excretion factors. Each of these uncertainties could cause underestimation or overestimation of per capita drug consumption. The mode of sampling and sampling frequency can cause error in estimation of drugs mass load and their consumption. Time-proportional sampling mode used in this study does not consider flow variations, which is less accurate than flow proportional sampling technique. Fifteen minutes of sampling frequency in this study could also cause uncertainty as short-term variations in drugs' loads can be easily ignored (Ort et al. 2010). Data interpretation in this study was also based on total 31 24-hour composite influent samples and five, four, and three 24-hour composite effluent samples at primary, Bardenpho, and MBR effluent points, respectively. Furthermore, the direct injection LC-MS/MS analytical protocol followed in this study might have resulted in higher LOD/LOR than solid phase extraction (SPE)-LC-MS/MS. The discrepancy between estimation of alcohol consumption in this study estimated by WBE and by epidemiological surveys could be because of in-sewer degradation of alcohol markers. The average human excretion rates of drugs have been used in this study for back calculation, which also adds to the uncertainty in estimation of drug's consumption.

3.2.4. Correlations

The Spearman's correlation rank analysis was performed to understand correlations between PNML of various drugs, wastewater inflow, and rainfall. Our findings showed that there was moderate correlations among DOAs' in influent. The correlations are marked as bold at $p < 0.05$ and correlations > 0.4, as shown in **Table S17**. The drugs and their metabolites were found to correlate with each other in the wastewater influent, as expected.. The mass load of nicotine markers were found to correlate with morphine. This study also revealed interdependence of methamphetamine and methadone consumption. The interdependence of methamphetamine and methadone could be because of concurrent usage of opioids and methamphetamine or use of methadone in methadone maintenance treatment (MMT) widely used to treat addiction (Radfar et al. 2016, Wang et al. 2015). The interdependence of morphine, cotinine, and hydrocotinine may reflect the population groups overlap since smokers have more opportunities to use morphine in illicit or licit ways, and since morphine can result from transformation of 6-acetylmorphine (metabolite of heroin) (Boleda et al. 2009).

4. Conclusion

This study revealed significant presence of biomarkers of alcohol and tobacco consumption and methamphetamine in wastewater influent, confirming the recent findings from the United Nations about their prevalence in this part of the world. Primary treatment was not effective, which indicated that conventional WWTPs will need to rely on secondary treatments for removal of DOAs. Although MBR is widely acknowledged as more expensive and advanced treatment, it did not report higher efficiencies than Bardenpho treatment in terms of removal of total DOAs. Total population estimated by hydrological parameters like COD and TP and human urine biomarkers, methadone and nicotine, showed good agreement with each other and with WWTP population estimate. Daily consumption of DOAs was calculated based on daily population estimates calculated from nicotine metabolites.

This study will not only aid WWTP operators but will also be of interest to environmental toxicologists, epidemiologists, and drug researchers. The findings confirm similar level of methamphetamine, cocaine, and MDMA consumption in New Zealand compared to Australia, and is consistent with recent seizure data and other research conducted in this part of the world. Although consumption patterns of DOAs were estimated in this study, due to level of uncertainties involved, associated with small numbers of samples and extrapolation factors used, more research is required before WBE could be used to evaluate public health interventions.

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Figure 1. Process diagram of the WWTP

Figure 2. Removal of selected DOAs by the secondary treatment

Figure 3. Population estimates based on hydrochemical and human urine biomarkers

Figure 4. Population normalised mass load (PNML) of DOAs in wastewater influent (mg/d/1000 person)

Table 1. Influent and Effluent DOAs' Concentrations in four seasons and Comparison with International Studies

DOA		Influent (ng/L)				International study	Effluent (ng/L)					International study
	Frequency of detection (%)	Minimum concentration	Maximum concentration	Mean± Standard deviation	Median (Number of composite samples above LOD)		Frequency of detection (%)	Minimum concentration	Maximum concentration	Mean± Standard deviation	Median (Number of composite samples above LOD)	
Amphetamine	7	<LOD	137[1]	134±4[1]	134[1] (2 out	<LOQ-4310[a], 27-	25	<LOD	383	-	- (1 out of 4)	n.d.-210[a], 6±2[c]

					of 28)	235[b], 43±5[c],15.8-143[d], 9.9±7.8[e]						, n.d.[d], 1.5 ± 1.8[e]
Methampheta mine	100	421	1268	935 ±62	942 (28 out of 28)	<LOQ-2000[a], 23-225[b], n.d.[c], <LOQ-11.7[d]	50	<LOD	59	58± 2	58 (2 out of 4)	0.4-350[a], n.d.[c], <LOQ-33.1[d]
Cocaine	11	<LOD	167	98± 18	98 (3 out of 28)	<LOQ-4700[a], 308-2667[b], 115± 8[c], <LOQ-156[d],	25	<LOD	286	-	- (1 out of 4)	<LOQ-530[a], 80± 8[c], <LOQ-2.14[d],

						56±19 [e]						27 ± 14 [e]
Benzoylecgonine	11	<LOD	184	127 ±10	127 (3 out of 28)	5-7500 [a], 729-3642 [b], 241± 29 [c], 157-3020 [d], 186± 59 [e]	25	<LOD	85	-	- (1 out of 4)	<LOQ-1500 [a], 471 ±73 [c], <LOQ-210 [d], 88 ± 83 [e]
Cotinine	100	717	2615	1,800±274	1985 (28 out of 28)	145-2680 [d]	100	41	70	54± 12	53 (4 out of 4)	5.74 - 44.2 [d]
Hydroxycotinine	100	2029	7966	5,000±512	5317 (28 out	-	0	<LOD	<LOD	<LOD	<LOD (0 out of 4)	-

					of 28)							
Nicotine	100	1082	14, 586	3,000±1063	2619 (28 out of 28)	555-6410 [d]	0	<LOD	<LOD	<LOD	<LOD (0 out of 4)	23.1 - 46.8 [d]
Ethyl sulfate	100	4, 448	13,719	8300 ±1134	7751(28 out of 28)	5500-32500 [b], 19200 [f], 1460-19850 [g]	0	<LOD	<LOD	<LOD	<LOD (0 out of 4)	<LOD [g]
Morphine	100	108	334	223 ±9	228 (28 out of 28)	28-1007 [a], 61-370 [b], 30±5 [c], 62.4–363 [d], 294±83 [e]	0	<LOD	<LOD	<LOD	<LOD (0 out of 4)	12-929 [a], 48±5 [c], n.d.-59 [d], 56 ± 25 [e]

Methadone	100	15	80	29±5	26 (28 out of 28)	2.6-1531 [a], 30-383 [b], 28±0.4 [c], <LOQ-54.6 [d], 52±16 [e]	100	19	28	23±4	22 (4 out of 4)	1.4-732 [a], 43±0.8 [c], <LOQ-36.8 [d], 37 ± 9 [e]
EDDP	100	32	71	53±5	54 (28 out of 28)	n.d.-1029 [a], 50-197 [b], 75±1 [c], 11.8-70.2 [d], 128±20 [e]	100	35	50	41±7	39 (4 out of 4)	2.6-1150 [a], 106 ±4 [c], 16.3-192 [d], 123 ± 25 [e]
Codeine	100	317	1140	753 ±49	792 (28 out	1.3-3973 [a], 513±25 [c],	100	80	145	104 ±28	96 (4 out of 4)	3-1502 [a], 795 ±32

					of 28)	262± 51 [e]						[c], 149 ± 31 [e]
MDMA	11	<LOD	26[1]	24± 4[1]	24 (3 out of 28) [1]	<0.5-598 [a], 23-287 [b], 131± 2 [c], 1.09-62.5 [d], 6.8±7.7 [e]	25[1]	<LOD	20[1]	-	- (1 out of 4)	<LOD-376 [a], 67± 5 [c], <LOQ-62.3 [d], 2.4 ± 1.7 [e]

[a] (Yadav et al. 2017); [b] (Mastroianni et al. 2017); [c] (Yargeau et al. 2014); [d] (Subedi and Kannan 2014); [e] (Terzic et al. 2010); [f] (Mastroianni et al. 2014); [g] (Andrés-Costa et al. 2016); [1]A concentration above the LOD but below LOR, is included if it is greater than the midpoint between the LOD and LOR (i.e. (LOD + LOR)/2)

Table 2. Seasonal Variation of DOAs in Influent (Mean ± Standard Deviation)

Influent DOA	Spring (ng/L)	Summer (ng/L)	Autumn (ng/L)	Winter (ng/L)	Heavy rainfall (ng/L)
Amphetamine	<LOD	<LOD	134± 4[1]	<LOD	187[1]
Methamphetamine	943±251	932±288	1,008±203	857±238	542±132
Cocaine	<LOD	110±81	<LOD	85	183
Benzoylecgonine	<LOD	135±70	<LOD	120	1,780
Cotinine	1,933±417	2,126±521	1,841±221	1,472±458	962±351
Hydroxycotinine	5,117± 1,048	5,614± 1,781	5,110± 649	4,371± 1,522	2,590± 815
Nicotine	2,863±654	4,509±4,515	2,315±357	2,208±676	1,145±497
Ethyl sulfate	8,183±2,297	8,799±2,228	9,333±3,794	6,707±1,763	3,431±1,322
Morphine	227±55	233±58	216±40	215±86	101±79
Methadone	28±7	29±7	35±20	23±3	17±6
EDDP	59± 13	52± 12	51± 6	48± 8	40± 6
Codeine	798±176	774±197	757±136	684±266	383±136
MDMA	21[1]	26[1]	<LOD	<LOD	<LOD
Total	**20,172**	**23,339**	**20,800**	**16,790**	**11,361**

[1]A concentration above the LOD but below LOR, is included if it is greater than the midpoint between the LOD and LOR (i.e. (LOD + LOR)/2)

Table 3. Comparison of DOAs consumption with previously published data for New Zealand (all values in mg/d/1000 person)

DOA	Present study (population ~70,000)	Christchurch (population ~375,000)	Auckland (population ~1,600,000)	Whangarei (population ~77,000)
Methamphetamine	484±73	240± 43[1]	411±50[1], 360±112[2]	900± 167[1]
Cocaine	94±34	15± 2[1]	57±4[1], 30.3±5.6[2]	4± 3[1]
MDMA	16±1	161± 55[1]	94±48[1], 60.2± 13.1[2]	7± 6[1]
Codeine	241±26	No data available	499±170[2]	No data available
Methadone	44±9	No data available	38 ±12.7[2]	No data available

[1](Chappell et al. 2017); [2] (Lai et al. 2017)

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Highlights:

- First comprehensive study in WWTP of New Zealand to delineate fate of illicit drugs
- Primary treatment was ineffective; MBR and Bardenpho showed ~95% removal of DOAs
- Biomarkers of alcohol, tobacco, and methamphetamine dominant in wastewater influent
- Amphetamine and cocaine detected at highest concentrations in wastewater effluent

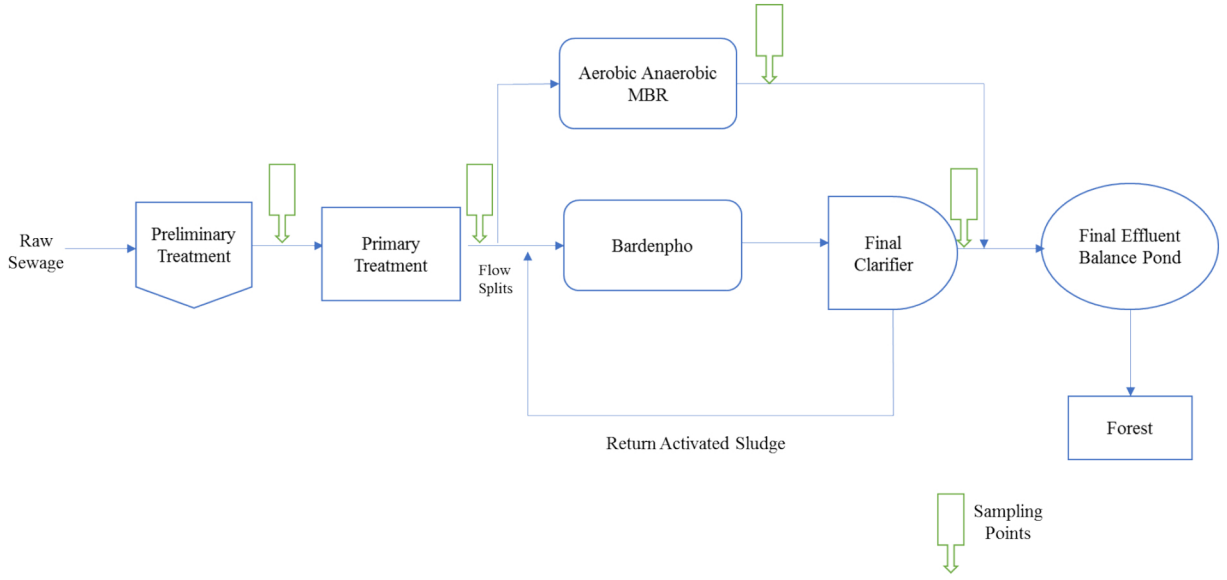
Aerobic Anaerobic MBR
Raw Sewage
Preliminary Treatment
Primary Treatment
Flow Splits
Bardenpho
Final Clarifier
Final Effluent Balance Pond
Forest
Return Activated Sludge
Sampling Points

Figure 1

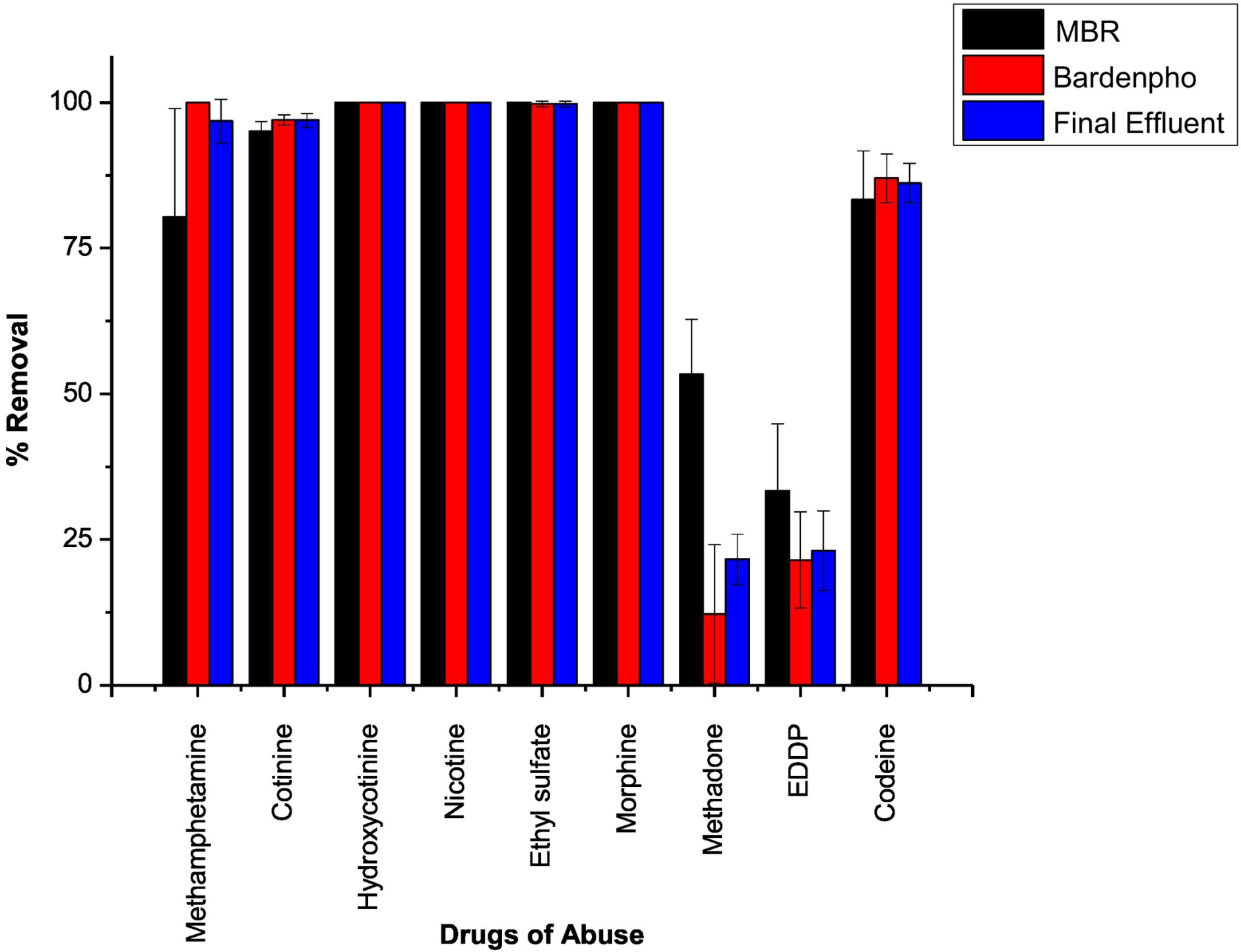
MBR
Bardenpho
Final Effluent
100
75
50
25
0
% Removal
Methamphetamine
Cotinine
Hydroxycotinine
Nicotine
Ethyl sulfate
Morphine
Methadone
EDDP
Codeine
Drugs of Abuse

Figure 2

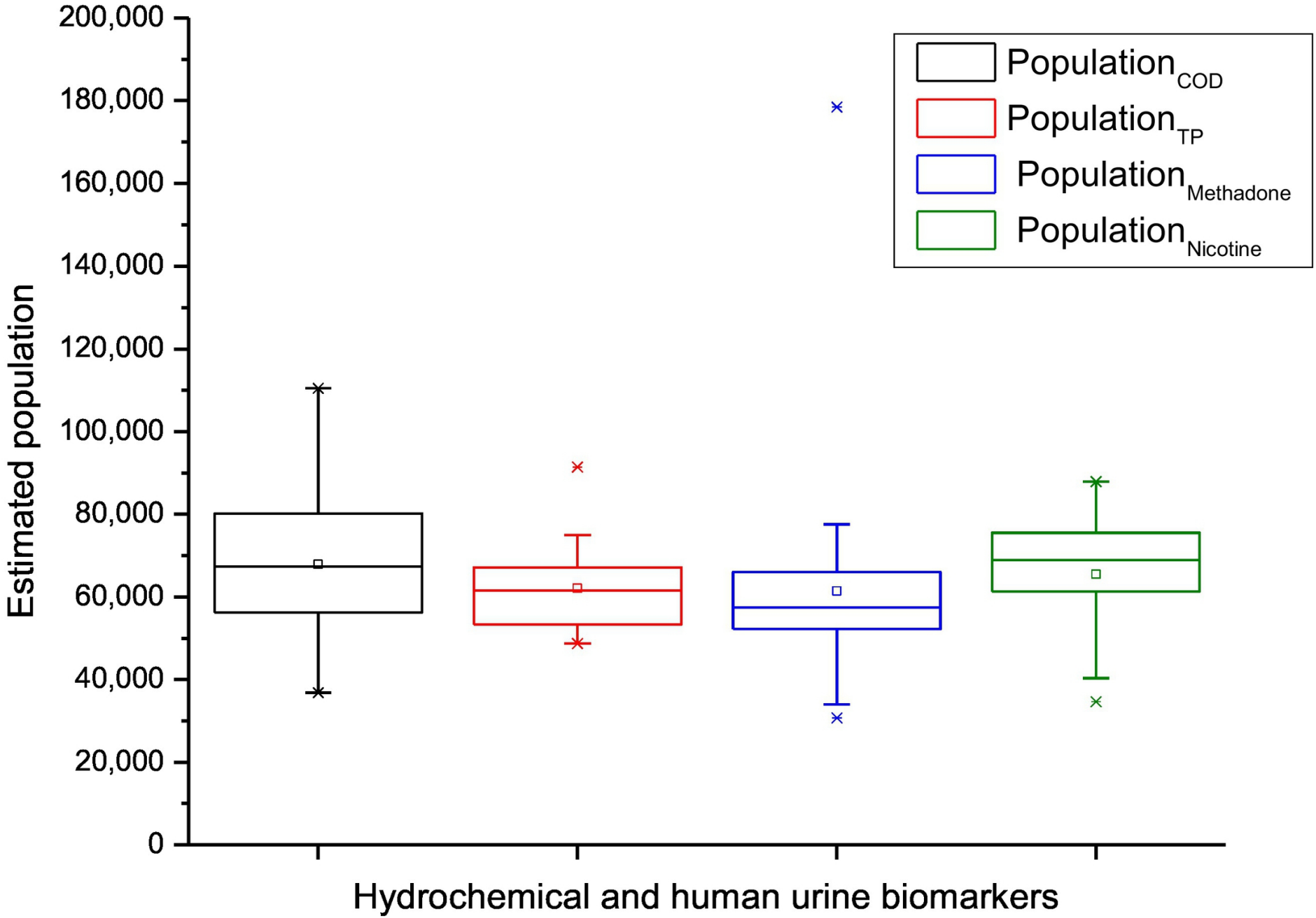
200,000
180,000
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
Estimated population
Population COD
Population TP
Population Methadone
Population Nicotine
Hydrochemical and human urine biomarkers

Figure 3

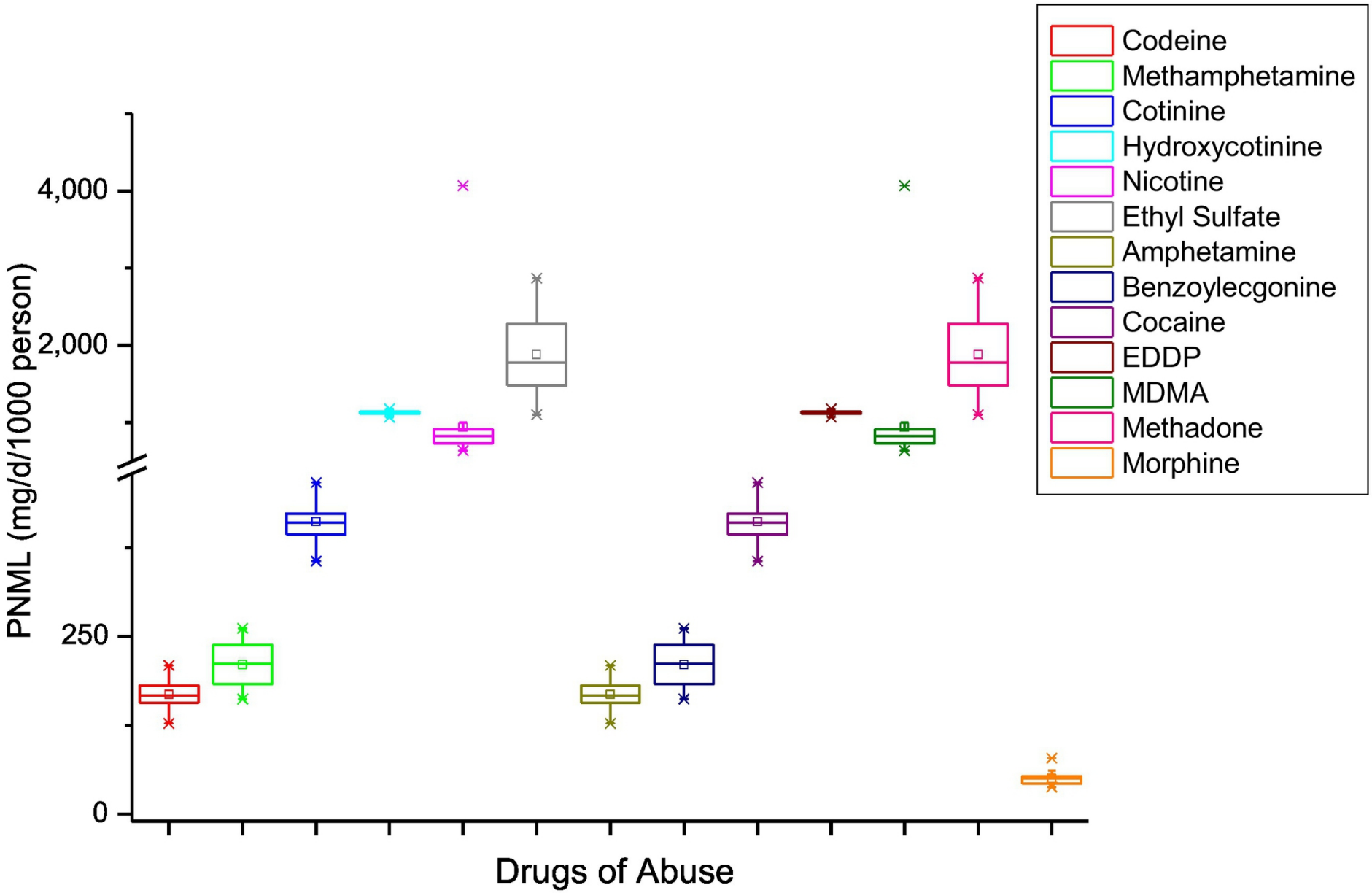
Codeine
Methamphetamine
Cotinine
Hydroxycotinine
Nicotine
Ethyl Sulfate
Amphetamine
Benzoylecgonine
Cocaine
EDDP
MDMA
Methadone
Morphine
4,000
2,000
250
0
PNML (mg/d/1000 person)
Drugs of Abuse

Figure 4